Naturwissenschaften (2005) 92:20–25
DOI 10.1007/s00114-004-0581-1

SHORT COMMUNICATION

Zaid Jumean · Tom Unruh · Regine Gries · Gerhard Gries

Mastrus ridibundus parasitoids eavesdrop on cocoon-spinning codling moth, *Cydia pomonella*, larvae

Received: 27 May 2004 / Accepted: 10 October 2004 / Published online: 18 November 2004

Abstract Cocoon-spinning larvae of the codling moth, *Cydia pomonella* L. (Lepidoptera: Olethreutidae) employ a pheromone that attracts or arrests conspecifics seeking pupation sites. Such intraspecific communication signals are important cues for illicit receivers such as parasitoids to exploit. We tested the hypothesis that the prepupal *C. pomonella* parasitoid *Mastrus ridibundus* Gravenhorst (Hymenoptera: Ichneumonidae) exploits the larval aggregation pheromone to locate host prepupae. In laboratory olfactometer experiments, female *M. ridibundus* were attracted to 3-day-old cocoons containing *C. pomonella* larvae or prepupae. Older cocoons containing *C. pomonella* pupae, or larvae and prepupae excised from cocoons, were not attractive. In gas chromatographic-electroantennographic detection (GC-EAD) analyses of bioactive Porapak Q extract of cocoon-derived airborne semiochemicals, ten compounds elicited responses from female *M. ridibundus* antennae. Comparative GC-mass spectrometry of authentic standards and cocoon-volatiles determined that these compounds were 3-carene, myrcene, heptanal, octanal, nonanal, decanal, (*E*)-2-octenal, (*E*)-2-nonenal, sulcatone, and geranylacetone. A synthetic 11-component blend consisting of these ten EAD-active compounds plus EAD-inactive (+)-limonene (the most abundant cocoon-derived volatile) was as effective as Porapak Q cocoon extract in attracting both female *M. ridibundus* and *C. pomonella* larvae seeking pupation sites. Only three components could be deleted from the 11-component blend without diminishing its attractiveness to *M. ridibundus*, which underlines the complexity of information received and processed during foraging for hosts. *Mastrus ridibundus* obviously "eavesdrop" on the pheromonal communication signals of *C. pomonella* larvae that reliably indicate host presence.

Z. Jumean · R. Gries · G. Gries (✉)
Department of Biological Sciences,
Simon Fraser University,
Burnaby, BC V5A1S6, Canada
e-mail: gries@sfu.ca

T. Unruh
Yakima Agricultural Research Laboratory,
USDA-ARS,
Wapato, WA 98951, USA

Introduction

Host-foraging hymenopterous parasitoids are often faced with a reliability–detectability problem. Semiochemicals (= message bearing chemicals) from the first trophic level are highly detectable and direct parasitoids to host habitat but are poor indicators of host presence. Semiochemicals from the host itself, in contrast, are reliable indicators of host presence, but are scarcely detectable to parasitoids because host insects express a low semiochemical profile (Vet and Dicke 1992; Stowe et al. 1995). Parasitoids learning to associate highly detectable host-habitat derived semiochemicals with highly reliable host-produced semiochemicals enhance their foraging effectiveness and increase their fitness (Vet and Dicke 1992; Hoffmeister and Roitberg 1997; Geervliet et al. 1998; Hoffmeister et al. 2000).

Potential hosts may maintain a low semiochemical profile, but cannot completely avoid emitting semiochemicals as intraspecific communication signals. These signals may become important cues for illicit receivers to exploit (Stowe et al. 1995; Haynes and Yeargan 1999) such as *Mastrus ridibundus* (Gravenhorst) (Hymenoptera: Ichneumonidae), a parasitoid of late instar/prepupal codling moth, *Cydia pomonella* L. (Lepidoptera: Olethreutidae) (Unruh 1997; Kuhlman and Mills 1999).

Cocoon-spinning *C. pomonella* larvae produce a pheromone that mediates aggregation/arrestment of pupation site-seeking larvae (Duthie et al. 2003). Conceivably, *M. ridibundus* may eavesdrop on the communication among *C. pomonella* larvae by exploiting the larval pheromone as a reliable host-derived kairomone.

Our objectives were to: (1) test the hypothesis that semiochemicals from cocoon-spinning *C. pomonella* larvae attract host-foraging female *M. ridibundus*; (2) determine the semiochemical source and longevity; (3) identify the semiochemicals; and (4) investigate whether

M. ridibundus eavesdrop on pheromonal communication of cocoon-spinning *C. pomonella* larvae.

Methods

Experimental insects

Cydia pomonella

Trays containing ~1,000 larvae (Sterile Insect Release Program facility, Osoyoos, BC, Canada) were kept in glass aquaria at 15°C and a 16L:8D photoperiod. To generate experimental test stimuli, groups of five final instars were removed from the diet and allowed to cocoon on 2.5×2.5-cm corrugated cardboard (Shippers Supply, Richmond, BC, Canada) placed in Petri dishes. If test stimuli consisted of cocoons, larvae were excised from cocoons 1.5 h before bioassays.

Mastrus ridibundus

Cydia pomonella hosts parasitized with *M. ridibundus* (originally collected in Kazakhstan; Unruh 1997) were shipped in corrugated cardboard rolls from the USDA-Agricultural Research Station, Wapato, Wash., USA. Rolls were kept in Plexiglas cages (40×30×30 cm) at 20–23°C, 30–50% RH, and a 16L:8D photoperiod. Emergent *M. ridibundus* were sustained with a 10% honey–water solution and a water-soaked cotton wick ad libitum. Naive, 3- to 14-day-old females of unknown mating status were isolated singly in plastic cups (4×4 cm) 1 h before experiments and bioassayed once.

Acquisition and analyses of volatiles

To collect airborne volatiles from cocoon-spinning larvae, 300 fifth-instar larvae were placed in a Pyrex glass chamber (15.5 ID×20 cm), and aerated for 72 h. A water aspirator drew purified air at 2 l/min through the chamber and a downstream glass column (140×10.1 mm ID) filled with Porapak Q (50–80 mesh, Waters, Milford, Mass., USA). Volatiles were eluted from Porapak Q with 3 ml of redistilled pentane:ether (95:5). The eluent was concentrated under a stream of nitrogen adjusting the volatile extract so that 1 μl was equivalent to ten cocoon-spinning larvae hour equivalents (10 CSLHE = volatiles released from ten cocoon-spinning *C. pomonella* larvae during 1 h). Aliquots of 20 CSLHE of Porapak Q extract were analyzed by gas chromatographic-electroantennographic detection (GC-EAD) and GC-mass spectrometry as previously described (Gries et al. 2002).

Olfactometer bioassays

Y-tube olfactometer bioassays

Responses of female *M. ridibundus* to test stimuli were bioassayed in vertically oriented Y-tube Pyrex glass olfactometers employing equipment and procedures as previously described (DeLury et al. 1999).

Experiments 1–4 tested potential sources of semiochemicals (larvae/prepupae or cocoon) derived from *C. pomonella*. Because semiochemical attractiveness resided with the cocoons, experiments 5–9 explored whether the age of cocoons affected their attractiveness. Experiments 10–11 then tested whether Porapak Q extracts of airborne cocoon semiochemicals were attractive (experiment 10), and whether the extract dose (experiment 11) affected the insects' response. Considering strong attraction of *M. ridibundus* to Porapak Q extract of cocoon semiochemicals (experiment 10), experiments 12–17 explored which of 11 candidate semiochemicals at natural ratios (see caption of Fig. 1) were essential for attractiveness of the synthetic blend (SB), by testing specific blends lacking classes of organic chemicals (e.g. aldehydes, ketones, or monoterpenes).

Abundant but EAD-inactive (+)-limonene was included in these experiments as it may have served as an organic solvent for minor blend components. Experiments 18–28 determined which individual compounds were essential components of SB, and experiments 29–30 tested SB lacking non-essential components against either Porapak Q cocoon extracts or the 11-component SB.

Petri dish olfactometer bioassay

Responses of pupation site seeking fifth-instar *C. pomonella* larvae were tested in two-choice Petri dish olfactometers (Duthie et al. 2003). Test stimuli were randomly assigned to one of two 4-ml vials fitted with modified Eppendorf tubes to prevent contact of larvae with test stimuli. For each replicate, one larva was placed in the center of the olfactometer, and its pupation site recorded 24 h later. Olfactometers were kept at 21–26°C in complete darkness.

Experiment 31 tested attractiveness/arrestment of the 11-component SB (at 200 CSLHE) to determine if *C. pomonella* larvae use SB semiochemicals as an aggregation pheromone.

Statistical analyses

Proportions of *M. ridibundus* females and *C. pomonella* larvae responding to test or control stimuli in bioassays were compared with the chi-square goodness-of-fit test with Yates' correction for continuity (α=0.05) (Zar 1999).

Results

GC-EAD analyses of Porapak Q extracts of cocoon volatiles revealed ten compounds that elicited responses from female *M. ridibundus* (Fig. 1). These compounds, plus the most abundant compound in Porapak Q extracts, were identified by comparative GC, GC-MS, and GC-EAD analyses of *C. pomonella*-produced and authentic standards, as follows: heptanal, sulcatone, myrcene, octanal, 3-carene, (+)-limonene, (*E*)-2-octenal, nonanal, (*E*)-2-nonenal, decanal, and geranylacetone.

Cocoons (3-day-old), but not larvae or prepupae of *C. pomonella*, attracted female *M. ridibundus* (Fig. 2; experiments 1–3). Attractiveness of cocoons was not enhanced by the presence of prepupae (Fig. 2; experiment 4), and depended upon the age of cocoons. Three-day-old cocoons containing late instar *C. pomonella* were attractive (Fig. 2; experiment 5), but 7- or 13-day-old cocoons containing pupal *C. pomonella* were not (Fig. 2; experiments 6, 7). Three-day-old cocoons were not more attractive than 7-day-old cocoons (Fig. 2; experiment 8), but were more attractive than 13-day-old cocoons (Fig. 2; experiment 9).

Porapak Q extracts of cocoon volatiles at aliquots of 1 CSLHE attracted *M. ridibundus*, whereas aliquots of 50 CSLHE did not (Fig. 2; experiments 10, 11). The 11-component SB at 1 CSLHE strongly attracted *M. ridibundus* (Fig. 2; experiment 12), whereas SB lacking either all aldehydes (Fig. 2; experiment 13), unsaturated aldehydes (Fig. 2; experiment 14), saturated aldehydes (Fig. 2; experiment 15), ketones (Fig. 2; experiment 16),

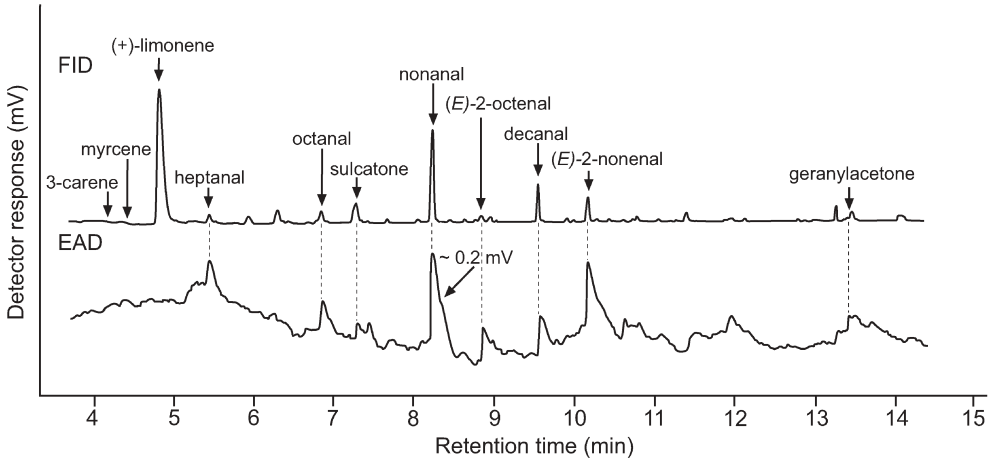

Fig. 1 Representative (n=6) flame ionization detector (FID) and electro antennographic detector (EAD: female *Mastrus ridibundus* antenna) responses to aliquots of 10 CSLHE of Porapak Q extract of airborne volatiles from *Cydia pomonella* cocoons (1- to 3-day-old). Note: (1) 10 CSLHE (cocoon-spinning larvae hour equivalents = volatiles released from 10 cocoon-spinning *C. pomonella* larvae during 1 h of cocoon-spinning activity); (2) chromatography: Hewlett-Packard 5890A gas chromatograph equipped with a GC column (30 m×0.25 mm ID) coated with DB-23 (J&W Scientific, Folsom, Calif., USA); FID temperature 240°C; temperature program: 50°C (1 min), 10°C per min to 200°C (hold for 5 min); (3) small quantities and poor chromatography of the monoterpene hydrocarbons 3-carene and myrcene on the polar DB-23 column resulted in distinct antennal responses to these compounds in only one out of six recordings; all other candidate pheromone components consistently elicited distinct antennal responses; the presence of 3-carene and myrcene was confirmed through GC-mass spectrometry of a concentrated (>200 CSLHE) extract, employing a GC column coated with DB-5; (4) FID profiles were similar in Porapak Q extracts of 6 separate aerations of cocoon-spinning larvae; (5) control aerations did not contain any of the EAD-active volatiles; and (6) although (+)-limonene was not EAD-active, it was the most abundant cocoon-volatile, as such could serve as a carrier for minor volatile components, and thus was included in bioassay experiments (see Fig. 2 and Fig. 3)

or monoterpenes (Fig. 2; experiment 17) was as unattractive as a pentane control. Deleting a single component from the 11-component SB in experiments 18–28 (Fig. 3) determined that an 8-component blend [heptanal, octanal, nonanal, decanal, (*E*)-2-octenal, sulcatone, geranylacetone, and 3-carene] is required to attract *M. ridibundus*. Neither Porapak Q cocoon extracts nor the 11-component SB attracted *M. ridibundus* more strongly than the 8-component SB (Fig. 3; experiments 29–30).

The 11-component SB also attracted/arrested pupation site seeking *C. pomonella* larvae (Fig. 3; experiment 31).

Discussion

Our data support the hypothesis that semiochemicals from cocoon-spinning *C. pomonella* larvae attract host-foraging *M. ridibundus*. This conclusion is based on the findings that *M. ridibundus* were attracted to cocoons containing *C. pomonella* late instar larvae (Fig. 2; experiment 5), and to Porapak Q extract of cocoon semiochemicals (Fig. 2; experiment 10). Moreover, attraction of *M. ridibundus* to *C. pomonella* cocoons (Fig. 2; experiments 1, 2), equal attraction to cocoons with or without *C. pomonella* inside (Fig. 2; experiment 4), and no attraction to excised *C. pomonella* prepupae (Fig. 2; experiment 3) suggest that the semiochemicals are associated with the cocoon.

While cocoon-derived semiochemicals have been implied as contact host recognition cues for parasitoids (e.g. Weseloh 1981; Bekkaoui and Thibout 1993), our data show that cocoon-derived airborne semiochemicals serve as long-range attractants sensu Kennedy[1] (1974) to *M. ridibundus*.

Response of *C. pomonella* larvae to the 11-component volatile blend (Fig. 3; experiment 31) reveals that one or more of these volatiles serve as an aggregation pheromone for *C. pomonella* larvae. Female *M. ridibundus* eavesdrop on the pheromonal communication of cocoon-spinning *C. pomonella* larvae to locate hosts. The pheromone is attractive to *M. ridibundus* in the larval cocoon-spinning stage, but no longer in the pupal stage (Fig. 2; experiments 5–9). Dissipation of the pheromone within 3 days after cocoon-spinning activity has ceased makes it a reliable indicator of host presence, allowing female *M. ridibundus*, as specialists of late instar *C. pomonella* larvae (Kuhlman and Mills 1999), to locate hosts at the proper stage of development.

For *C. pomonella* larvae, pheromone-based aggregation may represent a trade-off between procuring future mates (Duthie et al. 2003), and the risk of attracting parasitoids. Female *M. ridibundus*, for example, forage more frequently on trees with high rather than low

[1] An organism is attracted over long range if the distance to the stimulus exceeds a few body lengths of the organism. *Mastrus ridibundus* (<1 cm in body length) were attracted to test stimuli positioned >25 cm away.

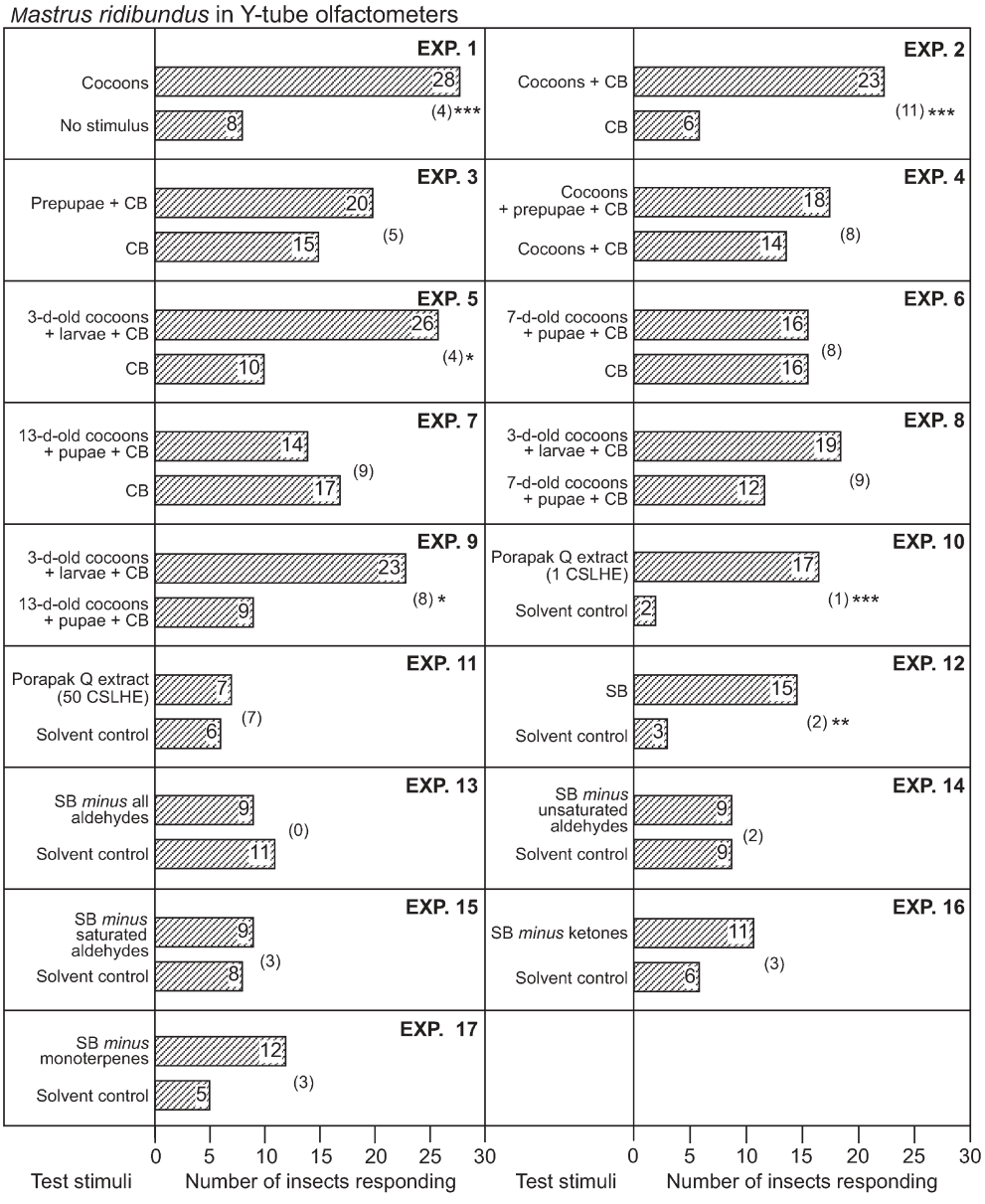

Fig. 2 Anemotactic response of female *Mastrus ridibundus* in Y-tube olfactometers to test stimuli consisting of: (1) five *Cydia pomonella* cocoons (3-day-old), five larvae/prepupae excised from cocoons or both (experiments 1–4); (2) five *C. pomonella* cocoons each containing either a larva/prepupa or pupa (experiments 5–9); (3) Porapak Q extracts of airborne cocoon-derived volatiles or a synthetic blend (SB) of 11 candidate semiochemicals (experiments 10, 11); or (4) SB lacking groups of organic chemicals (experiments 12–17). Number of insects responding to each stimulus given within bars; number of insects not responding in each experiment given in parentheses; asterisks indicate a significant response to a particular treatment; χ^2 test with Yates correction for continuity; **P*<0.025; ***P*<0.01; ****P*<0.005. Note: (1) Strips of corrugated cardboard (CB) served as pupation sites; (2) naive female *M. ridibundus* did not prefer CB over no stimulus in Y-tube olfactometers indicating that CB-derived odours did not affect the parasitoids decision, and that CB could be used as a control stimulus; (3) 1 or 50 CSLHE [cocoon-spinning larvae hour equivalents = volatiles produced by 1 or 50 cocoon-spinning *C. pomonella* larva(e) during 1 h of cocoon-spinning activity]; (4) SB consisted of 11 components tested at ratios and concentrations as determined by standard GC peak quantitation, and as found in 10 CSLHE, as follows: decanal (1.4 ng), nonanal (4.1 ng), octanal (0.94 ng), heptanal (0.85 ng), (*E*)-2-nonenal (1.0 ng), (*E*)-2-octenal (0.41 ng), geranylacetone (0.50 ng), sulcatone (0.81 ng), 3-carene (0.95 ng), myrcene (0.84 ng), (+)-limonene (10.0 ng); all chemicals were purchased from Aldrich (Milwaukee, Wis., USA) or Bedoukian (Danbury, Conn., USA) and were >95% chemically pure; (5) the same amount of solvent [pentane or pentane:ether (95:5)] (20 µl or 5 µl) was applied to treatment and control stimuli; and (6) solvents in test stimuli were allowed to evaporate for 30 s prior to bioassays

host densities (Bezemer and Mills 2001). However, if female parasitoids are egg-limited (Bezemer and Mills 2001), then the probability of parasitism may be lower for *C. pomonella* larvae cocooning in aggregation than for those cocooning singly.

Female *M. ridibundus* seem to have solved the reliability-detectability challenge of host signals by eaves-

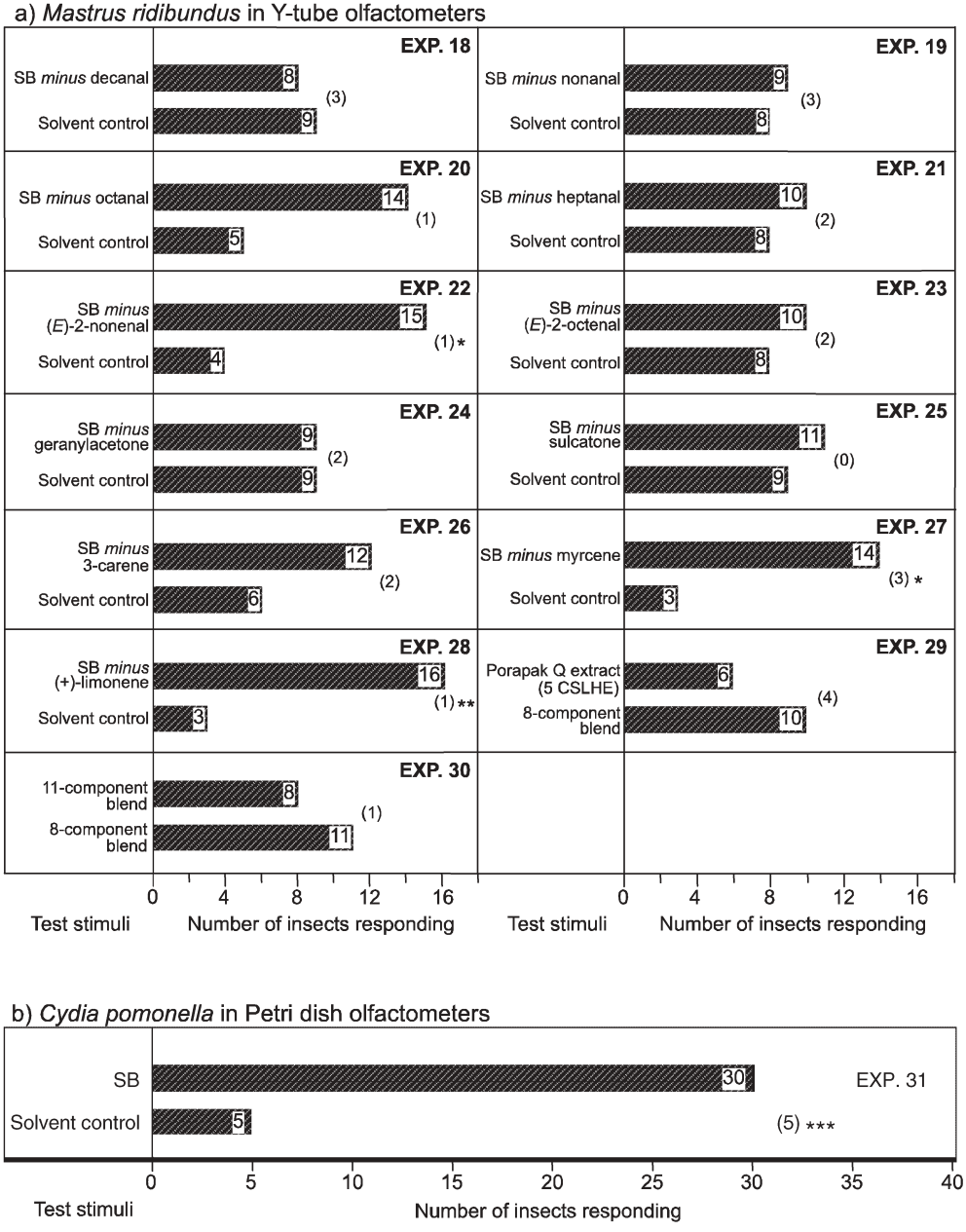

Fig. 3 a *Mastrus ridibundus*. Anemotactic response of females in Y-tube olfactometers to: (1) SB of candidate semiochemcals (see caption of Fig. 2) lacking individual components (experiments 18–28); (2) Porapak Q extract of airborne cocoon-derived volatiles at 5 CSLHE versus an 8-component blend of synthetic cocoon volatiles (experiment 29); or (3) 8- or 11-component blends of synthetic cocoon volatiles tested at ratios or concentrations as found in 10 CSLHE (see caption of Fig. 2) (experiment 30); **b** *Cydia pomonella*. Responses of fifth-instar larvae in Petri dish olfactometers (Duthie et al. 2003) to a SB of 11 candidate semiochemicals (see caption of Fig. 2) tested at ratios and concentrations as found in 200 CSLHE (experiment 31). Number of insects in experiments 18–31 responding to each stimulus given within bars; number of insects not responding in each experiment given in parentheses; asterisks indicate a significant response to a particular treatment; χ^2 test with Yates correction for continuity; $^*P<0.025$; $^{**}P<0.01$; $^{***}P<0.001$. Note: (1) the 8-component blend in experiments 29 and 30 was equivalent to the 11-component blend (see caption of Fig. 2) but lacked (*E*)-2-nonenal, myrcene, and (+)-limonene; (2) Porapak Q extract and 8-component blend in experiment 29 were tested at equivalent quantities; (3) the same amount of solvent was applied to treatment and control stimuli; (4) solvents in test stimuli were allowed to evaporate for 30 s prior to bioassays; (5) rationale to test 200 CSLHE (instead of a lower dose) in experiment 31 was based on the considerations that the test stimulus needed to remain effective during the 24-h test period for *C. pomonella* larvae (*M. ridibundus* females in experiments 1–30 typically responded within 2 min), and that the stimulus was applied only once, whereas cocoon-spinning larvae continuously replenish their pheromone; and (6) *C. pomonella* larvae also responded to lower doses of synthetic blends

dropping on the pheromonal communication of host larvae. Host pheromones are suitable signals for foraging parasitoids because pheromones tend to be detectable and reliable indicators of host presence (Wiskerke et al. 1993; Wertheim et al. 2003). The reliability of the cocoon-derived signal may be based on its complexity. Eight compounds from four classes of organic chemicals were required to elicit a strong behavioral response by *M. ridibundus*. It will now be intriguing to investigate whether the aggregation pheromone of *C. pomonella* larvae comprises an equally complex signal.

Acknowledgements Z.J. thanks Thelma Finlayson for a Finlayson Fellowship, and T.U. thanks the Washington Tree Fruit Research Commission for grants supporting parasitoid introduction. We also thank C. Lowenberger, M. Mackauer, P. Landolt, B. Roitberg, and G.J.R. Judd for review of the manuscript. The research was further supported by a grant from NSERC to G.G. Insects were maintained in SFU's Global Forest Quarantine Facility (GF-18–2000-SFU-6).

References

Bekkaoui A, Thibout E (1993) Role of the cocoon of *Acrolepiopsis assectella* (Lep., Hyponomeutidae) in host recognition by the parasitoid *Diadromus pulchellus* (Hym., Ichneumonidae). Entomophaga 38:101–113

Bezemer TM, Mills NJ (2001) Host density responses of *Mastrus ridibundus*, a parasitoid of the codling moth, *Cydia pomonella*. Biol Control 22:169–175

DeLury NC, Gries R, Gries G, Judd GJR, Khaskin G (1999) Moth scales-derived kairomones used by egg-larval parasitoid *Ascogaster quadridentata* to locate eggs of its host, *Cydia pomonella*. J Chem Ecol 25:2419–2431

Duthie B, Gries G, Gries R, Krupke C, Derksen S (2003) Does pheromone based aggregation of codling moth larvae help procure future mates? J Chem Ecol 29:425–436

Geervliet JBF, Ariëns S, Dicke M, Vet LEM (1998) Long-distance assessment of patch profitability through volatile infochemicals by the parasitoids *Cotesia glomerata* and *C. rubecula* (Hymenoptera: Braconidae). Biol Control 11:113–121

Gries R, Khaskin G, Gries G, Bennett RG, King GGS, Morewood P, Slessor K, Morewood WD (2002) (*Z*,*Z*)-4,7-Tridecadien-(*S*)-2-yl acetate: sex pheromone of Douglas-fir cone gall midge. J Chem Ecol 28:2283–2297

Haynes KF, Yeargan KV (1999) Exploitation of intraspecific communication systems: illicit signalers and receivers. Ann Entomol Soc Am 92:960–970

Hoffmeister TS, Roitberg BD (1997) To mark the host or the patch: decisions of a parasitoid searching for concealed host larvae. Evol Ecol 11:145–168

Hoffmeister TS, Roitberg BD, Lalonde RG (2000) Catching Ariadne by her thread: how a parasitoid exploits the herbivore's marking trails to locate its host. Entomol Exp Appl 95:77–85

Kennedy JS (1974) Changes in the patterning of behavioural sequences. In: Brown LB (ed) Experimental analysis of behaviour. Springer, Berlin Heidelberg New York, pp 1–6

Kuhlmann U, Mills NJ (1999) Exploring the biodiversity of Central Asia to assess specialized parasitoids for biological control of apple pests in Europe and North America. Integrated plant protection in orchards. IOBC WPRS Bull 22:1–6

Stowe MK, Turlings TCJ, Loughrin JH, Lewis WJ, Tumlinson JH (1995) The chemistry of eavesdropping, alarm, and deceit. Proc Natl Acad Sci USA 92:23–28

Unruh TR (1997) From Russia with love: new predators and parasites for control of tree fruit insect pests. Proceedings of the 93rd Annual Meeting of the Washington State Horticultural Association, pp 42–49

Vet LEM, Dicke M (1992) Ecology of infochemical use by natural enemies in a tritrophic context. Annu Rev Entomol 37:141–172

Wertheim B, Vet LEM, Dicke M (2003) Increased risk of parasitism as ecological costs of using aggregation pheromones: laboratory and field study of *Drosophila-Leptopilina* interaction. Oikos 100:269–282

Weseloh RM (1981) Host location by parasitoids. In: Nordlund DA, Jones RL, Lewis WJ (eds) Semiochemicals: their role in pest control. Academic, London, pp 79–95

Wiskerke JSC, Dicke M, Vet LEM (1993) Larval parasitoid uses aggregation pheromone of adult hosts in foraging behavior: a solution to the reliability-detectability problem. Oecologia 93:145–148

Zar JH (1999) Biostatistical analysis. Prentice Hall, Upper Saddle River, N.J.